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The role of Japanese macaques (*Macaca fuscata*) as endozoochorous seed dispersers on Kinkazan Island, northern Japan

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12 Abstract

13 We studied the characteristics of seeds within faeces, an important aspect of endozoochorous
14 seed dispersal, in Japanese macaques *Macaca fuscata* inhabiting Kinkazan Island, northern Japan.
15 We intermittently collected faecal samples from 1999 to 2009 (N = 1294) and examined the rate of
16 seed occurrence, species/life-form composition, number of seeds, and their intact rate . Seeds were
17 found within faecal samples during every month, but their characteristics changed monthly: the rate
18 of seed occurrence and the number of plant species within faecal samples were greater in summer
19 and fall, and the intact rate and number of intact seeds observed within single faecal samples were
20 also higher during these seasons than spring and winter. These results suggest that Japanese
21 macaques on Kinkazan act as seed dispersers in summer and fall and that they disperse intact seeds
22 into wider areas within the forest through defecation. During the study period, we observed seeds
23 from a total of 35 plant species from 22 families in our samples. In addition to those of woody plants,
24 we also observed seeds from as many as 13 herbaceous plants, for which sympatric sika deer
25 (*Cervus nippon*) have historically been considered the sole seed dispersal agents. The intact rate of
26 seeds was significantly negatively correlated with the seeds' mean cubic diameter, and this
27 relationship was strengthened for non-fleshy fruits. We also conducted regional comparisons of the
28 characteristics of defecated seeds in order to address whether regional variations in the diet of the
29 macaques affect their efficacy as seed dispersers, both in terms of quantity and quality. Macaques
30 living in the natural habitats of Kinkazan and Yakushima dispersed more seeds of tall tree species
31 than do macaques inhabiting the human-altered areas of in Shimokita and Kashima. The number of
32 plant species represented within single faecal samples also varied geographically, being greater in
33 Yakushima. This pattern likely resulted from Yakushima's warm-temperate climate, as the other
34 three study sites occur in the cool-temperate region. Our results suggest that the composition of
35 seeds dispersed by Japanese macaques are not rigid, but are determined instead by the vegetation

found in a given habitat.

Keywords: Japanese macaque; intact rate; Kinkazan; seed dispersal; temperate region

Introduction

Environmental and climatic conditions affect food availability, and can have an impact on dietary composition/diversity of given animals (Hill and Dunbar 2002). Studies aimed at comparing the feeding habits of a single mammal species across different geographical regions have revealed that fruit diversity in the diets of various mammals living at lower latitudes is greater than at higher latitudes, mainly because of differences in food availability and/or physical conditions (Virgòs et al. 1999; Zalewski 2004; Lozano et al. 2006; Zhou et al. in press). Investigating regional variation in the food habits of a particular species is therefore important for understanding its ecological plasticity, especially when considering wide-ranging mammals living in environments where the type and abundance of food resources are constantly changing (Rosalino and Santos-Reis 2009). With respect to seed dispersal, such regional variation in diet might affect that mammal's efficacy as a seed disperser, both in terms of quantity (the number of seeds dispersed) and quality (the number of plant species, proportion of intact seeds, and germination rate), leading to variation in forest ecosystem structuring. For example, frugivores inhabiting lower latitudes might disperse a larger number of intact seeds from a more diverse array of plant species than those inhabiting higher latitudes. Therefore, understanding the general role of a given mammal species as a seed disperser requires a comprehensive approach rather than surveying on a local scale.

Primates constitute one of the groups of frugivorous mammals inhabiting temperate regions. The diets of almost all cercopithecinae monkeys inhabiting temperate regions include at least some fruits (*Macaca mulatta*: Goldstein and Richard 1989; *M. cyclopis*: Su and Lee 2003; *M. fuscata*: Tsuji et al. 2006; *M. thibetana*: Zhao et al. 1991), and these animals can thus deposit seeds in locations far from

60 the seeds' origins. Compared to their sympatric avian seed dispersers, primates consume similar
61 amounts of fruit (Howe 1980; Son et al. 2007; Nakashima 2008), have larger gape sizes (Noma and
62 Yumoto 1997), have larger home ranges (1.3—6.4 ha for birds: Fukui 1995, <0.2 to >50 km^2 for
63 primates: Clutton-Brock and Harvey 1977), and exhibit longer retention times in the gut (15—30
64 minutes for birds: Murphy et al. 1993; Fukui 1996; 1.6—190 hours for primates: Lambert 2002;
65 Tsuji et al. 2010), all of which contribute to a wider dispersal of seeds. Thus, primates in temperate
66 regions are likely to strongly affect the spatial and genetic structure of plant populations and thereby
67 contribute to plant community dynamics.
68 (Fig. 1 should appear here)
69 Japanese macaques (*Macaca fuscata*) have the northern-most distribution of extant non-human
70 primates (30º 2'—41º 1' N). Previous studies of the role of Japanese macaques as endozoochorous
71 seed dispersers have been conducted mainly in the warm-temperate regions of Yakushima Island
72 (hereafter Yakushima). These studies have compiled lists of intact seeds observed within faeces
73 (Otani and Shibata 2000), estimated dispersal distance (Yumoto et al. 1998; Terakawa et al. 2009),
74 and examined the effects of: dispersal on seed germination/seedling growth (Yumoto et al. 1998);
75 seed/fruit characteristics on seed destruction (Otani and Shibata 2000); and, foraging patterns on the
76 topography of defecation sites (Tsujino and Yumoto 2009).
77 The habitat of Japanese macaques is roughly divided into two major climate regions: the
78 warm-temperate region dominated by evergreen forests in the southern part of their range and the
79 cool-temperate region dominated by deciduous forests in northern areas (Fig. 1). The food habits and
80 habitat utilisation (e.g., home range size, daily moving distance, and degree of frequent use) of
81 macaques differ between the two climate regions (Takasaki 1981; Agetsuma and Nakagawa 1998;
82 Maruhashi et al. 1998; reviewed by Tsuji 2010), largely because of differences in forest type. For
83 example, tall trees with fleshy fruits occur in greater abundance (Otani 2005) and density

(Maruhashi et al. 1998) in warm- than cool-temperate regions. Considering the wide variation of habitats throughout Japan, information from Yakushima represents only a small portion of the variation in seed dispersal patterns by Japanese macaques. The characteristics of seeds within faeces, an important aspect of endozoochorous dispersal, are likely to be affected by differences in the habitat structure and feeding behaviour of these animals. Thus, in order to gain a more general understanding of endozoochorous seed dispersal among Japanese macaques, regional comparisons between the cool- and warm-temperate regions are necessary. However, at present, few studies (Otani 2003) have addressed seed dispersal by macaques inhabiting cool-temperate regions.

The objectives of this study were to document the characteristics of seeds defecated by Japanese macaques inhabiting the cool-temperate Island of Kinkazan and to compare the results to those from other study sites. In terms of seed characteristics, we examined seed appearance, species/life-form composition, intact rate, the numbers of intact seeds and species contained within single faecal samples, and monthly changes in these parameters. Because macaques on Kinkazan feed on woody fruits primarily in the summer and fall (Tsuji et al. 2006), they most likely act as seed dispersers primarily during these two seasons, defecating large numbers of intact seeds from various plant species. In addition, we examined the relationship between seed size and their rate of destruction. On Yakushima, it is known that relatively larger seeds or seeds of non-fleshy fruits tend to be destroyed more often than smaller ones (Otani and Shibata 2000). We therefore expected that differences in forest type and consequent differences in plant species composition between the cool- and warm-temperate zones would lead to differences in this relationship.

Materials and methods

Study site and subject animals

Kinkazan (38º2’N, 141º4’E) is located 700 m from the Oshika Peninsula, northern Japan (Fig. 1).

The total area of the island is ca. 9.6 km^2, and the highest peak is 450 m a.s.l. The monthly mean air temperature on the island ranges from 2.5°C in February to 22.3°C in August. Based on the climate conditions of the island, the year was divided into four seasons: spring (March–May), summer (June–August), fall (September–November), and winter (December–February). On the island, deciduous forests of *Fagus crenata* dominate the higher elevations (>150 m), whereas a mixture of deciduous forests of *Carpinus* spp. (*C. tschonoskii* and *C. laxiflora*) and coniferous forests of *Abies firma* cover the lower elevations (<150 m). Kinkazan also harbours a forest of planted *Cryptomeria japonica* and patchy grassland communities of *Zoysia japonica* (Yoshii and Yoshioka 1949), the latter of which has expanded due to heavy grazing and seed dispersal by sympatric sika deer (*Cervus nippon*) (Takatsuki 2009). Approximately 200-250 Japanese macaques belonging to six troops (A, B_1, B_2, C_1, C_2, and D) inhabit the island (Izawa 2009).

Observation of the macaques

Observations of the monkeys of Troop A were conducted from June 2004 to May 2005 (88 days) (Tsuji et al. 2008). We recorded the foraging data of focal adult females (N = 14-17) with 1-min instantaneous sampling methods. Observations focused on foraging and handling techniques for fruit and seeds (Otani and Shibata 2000). Fruit species eaten by focal animals were classified into three categories, depending on the macaques' handling techniques for seeds: swallowed, crunched or discarded. Species classified as swallowed were those for which whole fruits were eaten and seeds were ingested and then passed in the faeces. For species classified as discarded, although whole fruits were eaten, only the flesh was ingested while the seeds were spat out. Crunched seeds were those that were crushed during mastication, although the processing technique for the flesh of the fruit varied by species. We could not identify a number of herbaceous plants (especially those of the family Gramineae) due to quick intake by the macaques.

132

133 Faecal sample collection and treatment

134 Fresh faecal samples were intermittently and opportunistically collected from the ground from
135 each troop between March 1999 and March 2009 (during 49 surveys in total), though we focused our
136 collection mainly within the home range of Troop A. Each faecal sample was placed into a plastic
137 bag and stored in a freezer at our field station. In the laboratory, each sample was thoroughly mixed
138 with water and rinsed through 0.5-mm sieves under fresh water. All contents were then placed into
139 plastic bottles with 70% alcohol and kept at room temperature until faecal analyses.

140

141 Analyses of faecal samples and regional comparisons

142 We removed all seeds from the bottled faecal samples and identified them to the species level.
143 Seed identification was based on a reference collection established in previous years as well as on
144 Nakayama et al. (2000). We classified the identified species into five life forms: tall trees (>5 m in
145 height), shrubs (<5 m), vines, hemi-parasites, and herbaceous plants. In addition, seeds were
146 classified into two fruit types: fleshy- and non-fleshy fruits.

147 We measured the longest axis (a_1), second longest axis (a_2), and third longest axis (a_3) of 10
148 randomly selected seeds for each plant species to the nearest 0.05 mm using vernier callipers. For
149 *Torreya nucifera*, we only measured one seed for each dimension due to a dearth of seeds in our
150 samples. From averaged values, we calculated the mean cubic diameter (*MCD*) of seeds (Otani and
151 Shibata 2000): $MCD = (a_1 \times a_2 \times a_3)^{1/3}$. After taking these measurements, we counted the total
152 number of seeds and the number of "intact seeds", defined as those seeds that appeared to be
153 complete, within each bottled sample. For plant species whose seeds were relatively large ($MCD \geq 1$
154 mm), we directly counted the numbers of both the total and intact seeds within the entire bottled
155 sample. Direct counts were difficult for smaller seeds ($MCD < 1$ mm), and we therefore sub-sampled

156 ca. 1 g (dry weight) of the bottle contents and counted the numbers of both whole and intact seeds
157 within the sub-sample. From this sub-sample, the numbers of both whole and intact seeds within the
158 entire bottled sample were estimated.
159 The evaluation of seed characteristics followed Kunz et al. (2008). We first calculated the
160 number of dispersal events (*DE*), which was defined as the number of faecal samples containing
161 seeds, for each month and for each plant species. Because plant species have different fruiting
162 periods and because we did not collect faecal samples in equal quantities across all months of the
163 study period (range: 10-374), we also calculated *DE* ratios (DE_r), obtained by dividing *DE* by the
164 number of faecal samples examined, for each month and for each plant species. To determine the
165 primary month(s) of *DE* for each species, we compared the number of intact seeds in a given month
166 to the seed's annual mean. Months during which the former was greater than the latter were defined
167 as main seed dispersal months . From this calculation, we obtained the primary seed dispersal
168 season(s). We also calculated an intact rate (*IR*) as a qualitative index of the efficacy of seed
169 dispersal (Koike et al. 2008) for each month and for each plant species. The *IR* was obtained by
170 dividing the total number of intact seeds in a given month by the total number of seeds in that
171 corresponding month, and the efficacy index was obtained by dividing the total number of intact
172 seeds of a given species during the entire study period by the total number of seeds of a given
173 species. Finally, we compared the DE_r, life-form composition, percentage of fleshy fruits, *MCD* of
174 seeds, and mean number of plant species within single faecal samples from Kinkazan to values from
175 three other study sites: Yakushima (Otani 2005), Shimokita (Otani 2003), and Kashima (Otani 2003).
176
177 Statistical analysis
178 To examine monthly changes in values of DE_r and *IR*, we employed binomial tests to compare
179 the observed DE_r (or *IR*) in a given month to the expected DE_r (or *IR*) (obtained from the annual

mean). To determine monthly changes in the total number of intact seeds, the number of intact seeds for each plant species, the number of species within single faecal samples, and to test the regional variation in the *MCD* across study sites, we used non-parametric Kruskal–Wallis analyses of variance (ANOVAs). Finally, to test the effects of the *MCD*, fruit type, and their interaction on *IR*, we used a generalized linear model (GLM). We treated the sum of intact seeds collected from whole faecal samples for each plant species as a dependent variable. The sum of all seeds of corresponding plant species collected from whole faecal samples was used as an offset term. We assumed the negative binomial distribution as an error structure. All data analyses were carried out using the statistical software R. 2.9.1 (R Development Core Team 2009). For all analyses, significance levels (α) were set at 0.05. We did not conduct statistical analyses for the regional comparisons, except for the *MCD*, because of small sample sizes at the other study sites.

(Table 1 should appear here)

Results

Observation of feeding behaviour

Fruit foraging was observed for a total of 659 h, during which the macaques foraged on the fruits of 29 woody plant species (17 families) and at least 8 herbaceous plant species (6 families) (Tsuji and Takatsuki in prep.). Consumed fruits were composed of 28 fleshy fruits and 9 non-fleshy fruits.

Monthly changes in seed occurrence and dispersal

A total of 1294 faecal samples were collected during 49 surveys (Table 1). The numbers of faecal samples collected in April (N = 10) and February (N = 19) were lower than those for other months, which may have affected the following results. The annual mean of the DE_r was 0.58 (747/1294), and more than 66000 seeds were collected from faeces (Table 1). Compared to the

annual mean, values of DE_r from June to December were significantly higher (binomial tests, $P < 0.05$), whereas the DE_rs from February to May were significantly lower ($P < 0.05$). In January, the DE_r did not significantly differ from the annual mean ($P > 0.05$; Table 1). The annual mean of the *IR* was 0.78 (66248/85042) (Table 1). Compared to the annual mean, the *IR* was significantly lower in March (binomial tests, $P < 0.05$), but significantly greater in June and from November to January ($P < 0.05$). In other months, the *IR* did not significantly differ from the annual mean ($P > 0.05$; Table 1).

Monthly changes in seed species composition

Table 2 shows the morphological characteristics and the handling techniques used for 36 different plant species extracted from the faecal samples, from which 35 species from 22 families were identified (Table 2). These species included 11 tall trees, 5 shrubs, 5 vines, 1 hemi-parasite, and 13 herbaceous plants. Seeds of shrubs were found during the entire year except for April. Seeds of herbaceous plants were found within faeces collected from May to December. Seeds of the hemi-parasite (*Viscum album*) were found in March and from November to February. Seeds of vines were found from June to January (Fig. 2). Identified plants included 20 fleshy-fruit and 15 non-fleshy-fruit species (Table 2). The total number of accumulated plant species was highest in October and November (18 plant species in each month) and lowest in February (2 species each) and April (none) (Fig. 2).

The seeds detected from the faecal samples contained many plant species whose seeds were swallowed by the macaques during our feeding observations, many of which being fleshy fruits (14/20 species). We also detected the seeds of many plant species whose seeds were crunched by the macaques, about half of which were non-fleshy fruits (7/15 species). Furthermore, seeds of two species (*Cornus kousa* and *Diospyros lotus*) that were discarded by the macaques during

228 observations were detected in faecal samples (Table 2).

229 Of the 35 identified plant species, six (*Vibrunum dilatum*, *Rosa multiflora*, *Vitis flexuosa*, *Malus*
230 *tchonoskii*, *Berchemia racemosa*, and *Oxalis corniculata*) exhibited significant monthly variation in
231 their number of intact seeds (Kruskal–Wallis tests, $P < 0.05$), and two (*Pourthiaea villosa* and
232 *Cornus kousa*) showed marginally significant monthly changes ($P < 0.10$). We observed seasonal
233 variation in the *DE* among species, and the number of intact seeds exhibited peaks during two main
234 seasons: summer (14 species) and fall (18 species) (Table 2).

235 (Table 2 and Fig. 2 should appear here)

236 Number of intact seeds and seed diversity within single faecal samples

237 The mean (± SD) total number of intact seeds within single faecal samples was 58 ± 176, and
238 this value exhibited significant monthly variation (Kruskal–Wallis test, $\chi^2 = 759.4$, $df = 10$, $P <$
239 0.001) (Table 3). The number of intact seeds was greatest in June and lowest in February, March, and
240 May. Of the 747 faecal samples that contained intact seeds, the mean seed diversity (i.e. number of
241 plant species represented) was 2.0 ± 0.9 (range:1-7) (Table 3). The mean seed diversity showed
242 significant monthly variation as well (Kruskal–Wallis test, $\chi^2 = 272.3$, $df = 10$, $P < 0.001$), and was
243 highest in June (4.1 ± 1.7) and lowest in May (1.5 ± 1.3), July (1.3 ± 0.6), and February (1.3 ± 0.5)
244 (Table 3).

245 (Table 3 should appear here)

246 Relationship between the intact rate and seed size/fruit type

247 The *MCD* of dispersed seeds was 3.0 ± 2.0 mm (range: 0.8–10.0) (Table 2). *IR* was
248 significantly affected by both the *MCD* (GLM: $z = -4.32$, $P < 0.001$) and by an interaction between
249 fruit type and the *MCD* ($z = 2.39$, $P = 0.017$) in which the effect of the *MCD* on the *IR* was
250 strengthened for non-fleshy fruits (Fig. 3).

251 (Fig. 3 should appear here)

252 Regional comparisons

253 A regional comparison revealed that the DE_r for Kinkazan (0.58) was lower than that for
254 Yakushima (0.99), Shimokita (0.93), and Kashima (0.79) (Table 4). The life-form composition of
255 plant species represented in faecal samples varied among the study sites such that, on Kinkazan, the
256 percentage of tall trees (31%) was similar to that on Yakushima (38%), and greater than the other
257 two sites (8% and 17%, respectively). The percentages of shrubs (17%) and vines (14%) represented
258 in samples from Kinkazan were lower than those from other study sites, whereas the percentage of
259 herbaceous plants (33%) was greater than that from other sites (Table 4). The percentage of fleshy
260 fruits observed in our Kinkazan samples (56%) was lower than those from the other three study sites
261 (Yakushima: 91%, Shimokita: 92%, Kashima: 89%). The *MCD* of seeds did not significantly vary
262 among the four study sites (Kruskal-Wallis test, $\chi 2 = 1.93$, $df = 3$, $P = 0.586$) (Table 4). In contrast,
263 although we were not able to test for the significance of this pattern, the mean seed diversity per
264 faecal sample was greatest on Yakushima (2.8 ± 1.4), followed by Kashima (2.4 ± 1.9) and Kinkazan
265 (2.0 ± 0.9), and was lowest on Shimokita (1.9 ± 0.8).

266 (Table 4 should appear here)

267 Discussion

268 Monthly changes in seed characteristics within faeces

269 In summer and fall (strictly from June to December), values of DE_r and *IR* were greater than
270 their annual means (Table 1), the seed diversity was high (Fig. 2), and the number of intact seeds
271 peaked for many plant species (Table 2). Furthermore, the numbers of intact seeds and seed diversity
272 were greater in summer and fall than spring and winter (Table 3). Thus, as we predicted, the role of
273 Japanese macaques as seed dispersers on Kinkazan appeared to be greater in summer and fall, during
274 which macaques dispersed intact seeds of various plant species more frequently and into wider areas
275 within the forest than during winter and spring.

276 The observed seasonal variation in seed dispersal can be attributed to seasonal changes in the
277 feeding behaviour of macaques on Kinkazan: in summer and fall, these animals spend a great deal of
278 time feeding on various kinds of fruits (ca. 20-60%) (Tsuji et al. 2006). The macaques ingest large
279 amounts of fruit to accumulate body fat (Tsuji et al. 2008) and may incidentally swallow the entire
280 fruit without adequately chewing it. In contrast, when fruit is less available during spring and winter,
281 macaques opportunistically feed on fruits remaining on the ground and/or on tree branches
282 (Nakagawa 1989; Tsuji et al. 2006), and may thus take the time to chew the contents well before
283 swallowing.
284
285 Plant species dispersed by macaques on Kinkazan
286 We found that macaques on Kinkazan defecate intact seeds of 35 species from 22 families ,
287 corresponding to 50.7% of all species of fruits on which this population is known to feed (69 species,
288 Izawa 2009). We detected seeds of more than half of all woody plants observed to be ingested during
289 behavioural observations (22 out of 29 species). Interestingly, the macaques also dispersed the seeds
290 of 13 herbaceous plants (Table 2). Before the present study, baboons (*Papio* spp.) were the only
291 known primate species to disperse seeds of herbaceous plants (Slater and du Toit 2002; Kunz et al.
292 2008). On Kinkazan, sympatric sika deer (*Cervus nippon*) are typically considered the primary
293 dispersers of herbaceous seeds (Takatsuki 2009). Because the biomass of the macaques on Kinkazan
294 (ca. 2500 kg as estimated by multiplying mean body weight (ca. 10 kg) by population size (ca. 250);
295 Izawa 2009) is much lower than that of deer (ca. 30000 kg; Takatsuki 2009), the amount of seeds
296 dispersed by macaques in each instance would likely be less than that by deer. However, the home
297 range size of macaques (ca. 3 km^2; Tsuji and Takatsuki 2009) is much larger than that of deer (<20
298 ha; Ito and Takatsuki 2009), and therefore, macaques would be expected to disperse seeds into wider
299 areas than would deer.

300 We also found that the seeds of several species whose fruits were crunched (example.g. the nuts
301 of Fagaceae) or discarded (*Cornus kousa* and *Diospyros lotus*) by the macaques were sometimes
302 detected in faecal samples (Table 2).However, since both the DE_r and *IR* of these species were lower
303 than those of species whose seeds were mainly swallowed, the contribution of macaques to the
304 dispersal of these seeds is likely negligible.
305
306 Relationship between seed size and intact rate
307 The vast majority of ingested seeds in our faecal samples were intact (annual mean *IR* = 0.78)
308 (Table 1), having a proportion much greater than values observed in captive Japanese macaques
309 (0.04 in Otani 2004; 0.36 in Tsuji et al. 2010). The variation in the intact rate of seeds between wild
310 and captive conditions can be attributed to a difference in the macaques' adherence/handling to the
311 plant seeds in each environment. The *IR* varied among plant species, and the *IR* and *MCD* were
312 negatively correlated such that larger seeds tended to be destroyed more frequently (Fig. 3), similar
313 to results obtained for forest guenons (*Cercopithecus* spp.; Gautier-Hion 1984). Because the
314 probability of seed destruction appears to be directly proportional to seed size, our results are
315 unsurprising. We also detected a significant interaction between *MCD* and fruit type such that the
316 effects of the seed size on the *IR* was greater for non-fleshy fruits (Fig. 3) The reason for this is
317 unclear, but we speculate that that seeds of non-fleshy fruits are not covered by a fleshy pulp, and the
318 risk of a given seed being destroyed might therefore increase in proportion to its size. In any case,
319 since masticatory seed mortality may affect dispersal efficiency, it is necessary to consider the
320 possible effects of fleshy pulp on seed dispersal characteristics.
321
322 Regional comparisons
323 The annual DE_r on Kinkazan was lower than values observed at the other study sites (Table 4).

Our larger sample size may have contributed to this difference, but other possible causes remain unclear. Our study on Kinkazan and the study on Yakushima by Otani (2003), both of which were conducted in natural forest environments though in different climates (cool-temperate versus warm-temperate, respectively), produced similar results in the percentage with which macaques ingested seeds of tall tree species (31% and 38%, respectively). These values are higher than those reported for Shimokita (8%) and Kashima (17%), both of which are located in cool temperate regions has and have been affected by human activity (Otani 2003). Our results reveal that the composition of seeds dispersed by Japanese macaques are not rigid, but are instead likely determined by the vegetation within a given habitat, as noted by Corlett and Lucas (1990). Because the size of intact seeds within faecal samples was similar across the four study sites, every plant whose seeds lie within the 2–3-mm threshold has the potential to be dispersed intact by macaques, regardless of its life form. Therefore, macaques differentially contribute to forest structure across different habitats.

The mean seed diversity per faecal sample varied from 1.9 to 2.9 across the four study sites, and was greater at lower latitudes (i.e. the warm temperate regions of Yakushima) than at higher latitudes (i.e. the cool temperate regions of Shimokita, Kinkazan, and Kashima). In general, macaques in cool temperate regions utilise their home ranges less intensively thanthose in warm temperate regions (Maruhashi et al. 1998). Thus, in warm temperate regions, the fruit contents of a single feeding bout at one feeding site might be packaged in one single faecal sample, resulting in a greater number of plant species within individual faeces samples.

On Yakushima, macaques disperse plant seeds not only through defecation, but also through discarding behaviour (Yumoto et al. 1998; Otani and Shibata 2000). Discarded seeds exhibited relatively large *MCD* values according to that study (7.3 ± 4.5 mm, range: 2.6—15.3; Otani and Shibata 2000). On Kinkazan, in contrast, we rarely observe seed discarding behaviour, except with seeds of *Cornus kousa* (*MCD*: 4.5 mm) and *Diospyros lotus* (*MCD*: 7.8 mm). Because the number of

larger-seeded plants is lower in cool temperate than warm temperate regions (Otani 2005), situations during which macaques might discard larger seeds are expected to be rare in the former. Furthermore, , many of the larger seeds on Kinkazan were nuts, which were [always OR typically OR generally OR usually OR most often OR etc] crunched rather than swallowed. Considering that the dispersal distance attained through discarding behaviour is shorter than that that via defecation (Yumoto et al. 1998), regional differences in the handling of seeds would likely influence seeds shadows. In general, the home range sizes of macaques in cool temperate regions are greater than those in warm temperate regions (Takasaki 1981). In combination with the fact that macaques in cool temperate regions utilise their home range less intensively (Maruhashi et al. 1998), this suggests that macaques in cool temperate regions should disperse seeds more widely and sparsely than macaques in warm temperate regions. Such variation in the patterns of dispersal would ultimately lead to differences in the forest structures of the two climate regions. To confirm these predictions, future studies should collect quantitative data regarding feeding behaviour and defecation, conduct experiments on seed germination/growth in both cool and warm temperate regions, and make regional comparisons between the two climate types.

In this study we demonstrated regional variation in the role of Japanese macaques as seed dispersers. Our results can be generalized not only to other primate species but also to other mammalian seed dispersers, especially concerning wide-ranging mammals (e.g. genets (Virgòs et al. 1999), martens (Zalewski 2004), baboons (Hill and Dunbar 2002)) living in environments in which the type and abundance of food resources are constantly changing (Rosalino and Santos-Reis 2009).

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Figure Legends

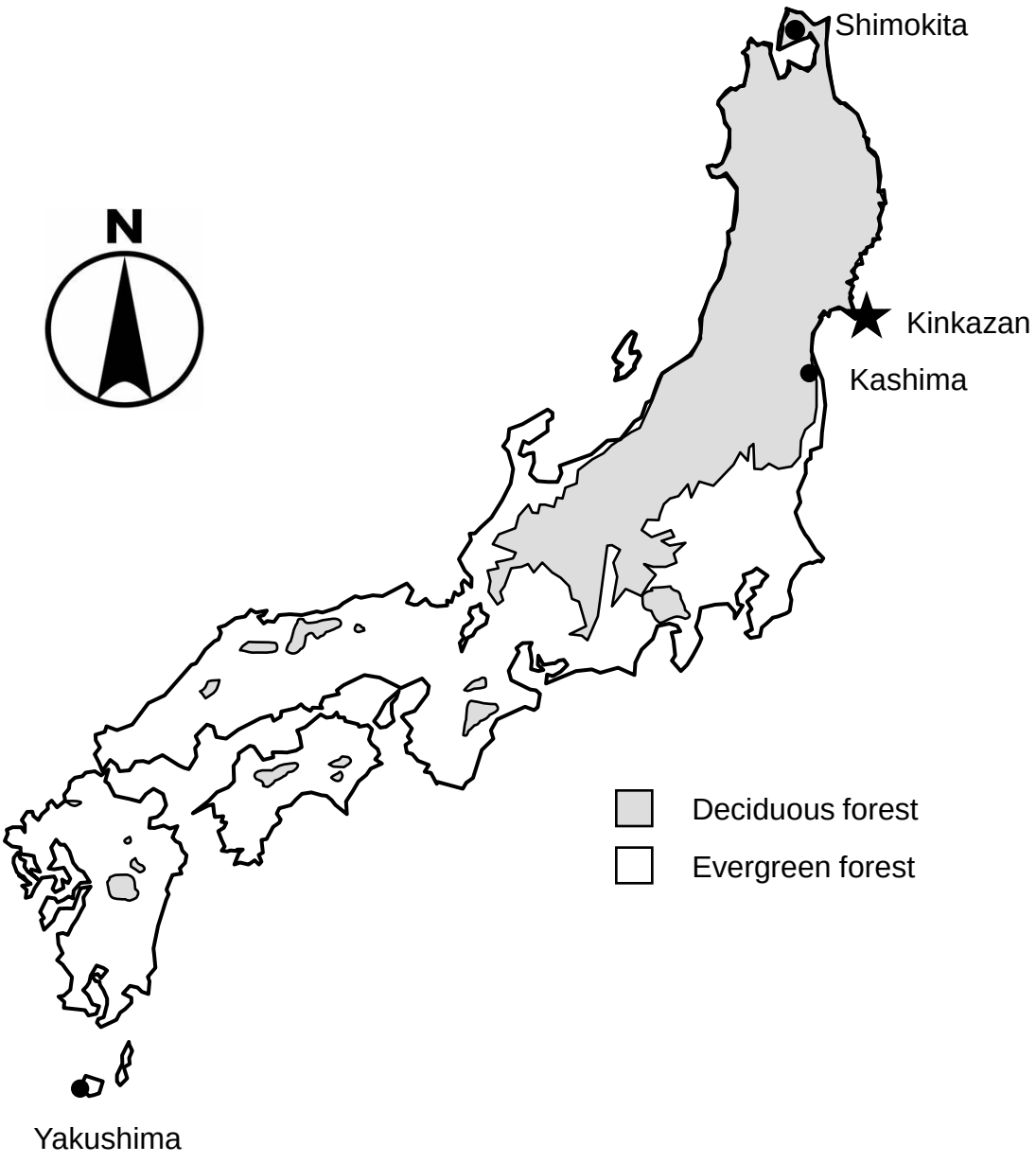
Figure 1. Distribution of the two main vegetation types in the natural habitats of Japanese macaques (■: deciduous forest, □: evergreen forest) (from Tsuji 2010) and locations of the four study sites (Shimokita, Kinkazan, Kashima, and Yakushima). Subalpine/alpine zones are omitted from the map for convenience. The black star indicates the location of Kinkazan, where the present study was conducted.

Figure 2. Monthly changes in the accumulated number of plant seeds found within faecal samples in a given month. Life-form composition (categorized into tall trees, shrubs, vines, hemi-parasites, and herbaceous plants) is also shown. Unidentified species are omitted. The whole year was separated into four seasons: spring: March to May; summer: June to August; fall: September to November; and winter: December to February. Numbers in parentheses represent the number of collected faecal samples.

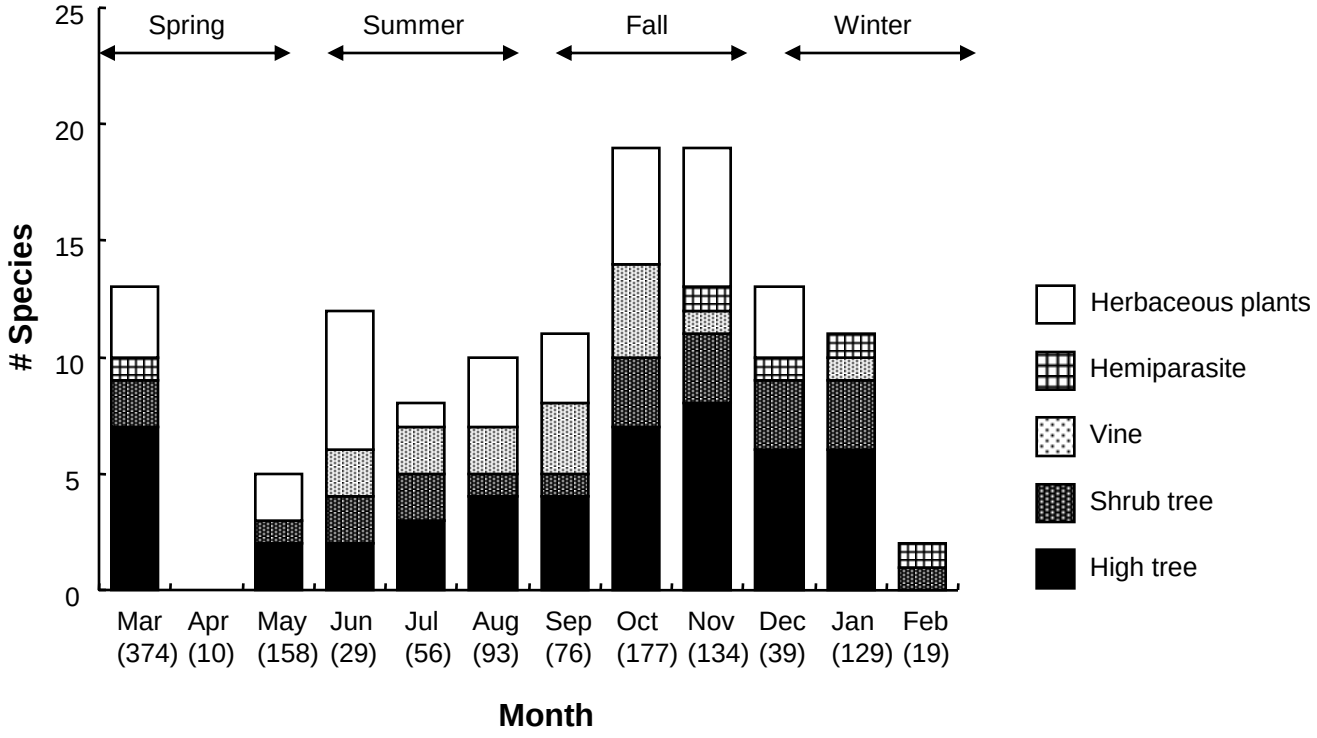
Figure 3. The relationship between mean cubic diameter (*MCD*) of seeds (mm) and their intact rate (%). ●: fleshy fruit, ○: non-fleshy fruit.

Figure

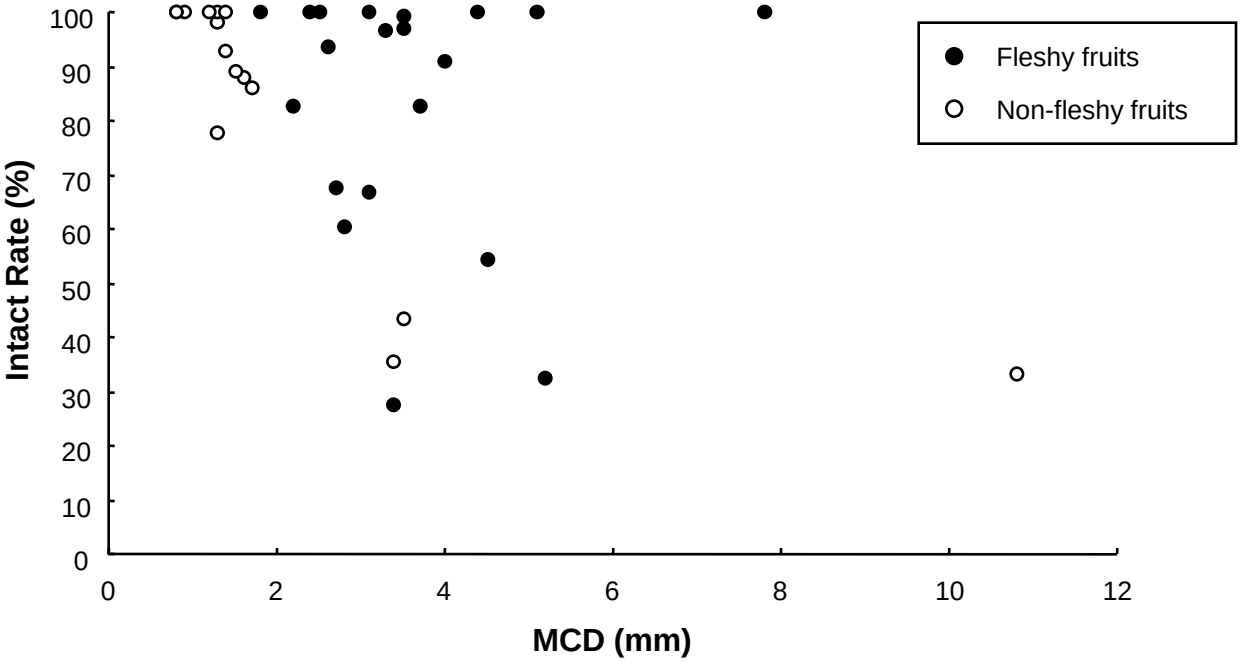
Tsuji et al. Figure 1



Tsuji et al. Figure 2



Tsuji et al. Figure 3



Table

Table 1. Monthly changes in dispersal event and total numbers of seeds defecated by the Japanese macaques on Kinkazan, northern Japan.

Month	# fecal sample	*DE*	DE_r [a]	Σ of seeds	Σ of intact seeds	*IR*
Spring						
March	374	83	0.22^{***}	3209	1977	0.62^{***}
April	10	1	0.10^{**}	2	0	0.00^{NS}
May	158	21	0.13^{***}	373	306	0.82^{NS}
Summer						
June	29	29	1.00^{***}	22327	2094	0.94^{***}
July	56	45	0.80^{***}	2806	2006	0.72^{NS}
August	93	90	0.97^{***}	9408	7743	0.82^{NS}
Fall						
September	76	56	0.74^{**}	3013	2365	0.79^{NS}
October	177	169	0.96^{***}	9439	6824	0.72^{NS}
November	134	130	0.97^{***}	22848	20198	0.88^{***}
Winter						
December	39	37	0.95^{***}	9535	8333	0.87^{*}
January	129	82	0.64^{NS}	2062	1122	0.54^{***}
Feburuary	19	4	0.21^{*}	20	20	1.00^{NS}
Annual mean	1294	747	0.58	85042	66248	0.78

[1]Numbers in parentheses are percentage of total fecal samples. Results of binomial test are also shown.
***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, NS: not significant ($P > 0.05$).
DE: dispersal event, DE_r: rate of dispersal event, *IR*: intact rate

Table

Table 2. Seeds found within fecal samples of Japanese macaques on Kinkazan, northern Japan (1999-2009).

Food plant species	Life form	*SHT*	*MCD* (mm, Mean ± SD)	*DE*	*DE*$_r$	# intact seeds (Mean±SD)	Months in which intact seeds appeared[a]	*P*-value[b]	Main season(s) in seed appearance[c]	Σ of seeds	Σ of intact seeds	*IR*
Taxaceae												
Taxus cuspidata	high tree	C	3.1 ± 0.4	1	0.00	1.0	**Mar**	—	Sp	1	1	1.00
*Torreya nucifera**	high tree	C	10.8	3	0.00	0.3 ± 0.6	**Sep**, Nov	0.157	F	3	1	0.33
Betulaceae												
Carpinus spp.*	high tree	C	3.5 ± 0.4	117	0.09	2.8 ± 6.2	**Mar**, Jun, Jul, Aug, Oct, Nov, **Dec**, Jan	0.270	Sp, W	756	328	0.43
Ulmaceae												
*Zelkova serrata**	high tree	C	3.4 ± 0.3	66	0.05	1.9 ± 3.0	Mar, **Aug**, Oct, **Nov**, Jan	0.092	Su, F	354	125	0.35
Loranthaceae												
Viscum album	hemi-parasite	S	4.0 ± 0.4	75	0.06	13.5 ± 29.2	Mar, Nov, **Dec**, Jan, Feb	0.895	W	1113	1013	0.91
Schisandraceae												
Schisandra nigra	vine	S	4.4 ± 0.3	49	0.04	17.1 ± 25.0	**Sep**, Oct	0.297	F	838	838	1.00
Actinidiaceae												
Actinidia arguta	vine	S	1.4 ± 0.1	12	0.01	30.5 ± 33.0	**Jul**, Aug	0.381	Su	394	366	0.93
Rosaceae												
Rosa multiflora	shrub	S	2.2 ± 0.3	221	0.17	83.9 ± 196.6	Mar, May, Oct, **Nov**, **Dec**, Jan, Feb	**< 0.001**	F, W	22475	18542	0.83
Pourthiaea villosa	shrub	S	2.7 ± 0.3	85	0.07	10.5 ± 18.1	Mar, Sep, **Oct**, Nov, **Dec**, Jan	0.088	F, W	1325	893	0.67
Prunus spp.	high tree	C	5.2 ± 0.2	15	0.01	1.7 ± 2.6	**Jun**, Jul	0.688	Su	80	26	0.33
Rubus microphyllus	shrub	S	1.3 ± 0.1	13	0.01	65.2 ± 147.8	**Jun**, Jul	1.000	Su	848	848	1.00
Sorbus japonica	high tree	S	3.1 ± 0.5	13	0.01	4.7 ± 5.6	Sep, **Oct**, **Nov**, Dec, Jan	0.353	F	91	61	0.67
Malus tschonoskii	high tree	S	3.5 ± 0.3	74	0.06	8.7 ± 9.7	Mar, **Sep**, **Oct**, **Nov**, Dec, Jan	**< 0.001**	F	665	644	0.97
Leguminosae												
*Amphicarpaea bracteata**	herbaceous	C	1.6 ± 0.2	33	0.03	4.2 ± 8.0	**Jul**, Aug, **Sep**, **Oct**, Nov, Dec	0.310	Su, F	158	139	0.88
*Desmodium podocarpum**	herbaceous	S	5.1	1	0.00	1.0	**Nov**		F	1	1	1.00
Rutaceae												
Zanthoxylum piperitum	shrub	C	2.8 ± 0.2	49	0.04	1.0 ± 1.0	Mar, Sep, **Oct**, **Nov**, **Dec**	0.563	F, W	81	49	0.61
Aquifoliaceae												
Ilex macropoda	high tree	S	2.6 ± 0.4	55	0.04	11.3 ± 17.2	**Mar**, Aug, Nov, Dec, Jan	0.147	Sp	665	622	0.94
Rhamnaceae												
Berchemia racemosa	vine	S	3.7 ± 0.2	126	0.10	64.8 ± 62.5	Jun, Jul, **Aug**, Sp	**0.001**	Su	9897	8165	0.83
Vitaceae												
Vitis flexuosa	vine	S	3.5 ± 0.3	79	0.06	26.0 ± 29.9	**Sep**, Oct, Nov, Jan	**0.023**	F	2073	2054	0.99
Cornaceae												
Swida macrophylla	high tree	C	3.4 ± 0.1	124	0.10	1.4 ± 2.9	Mar, Jul, Sep, **Oct**, **Nov**, Dec, Jan	0.193	F	211	174	0.83
Cornus kousa	high tree	D	4.5 ± 0.4	50	0.04	13.0 ± 25.2	Aug, **Sep**, Oct	0.071	F	1199	650	0.54
Araliaceae												
Acanthopanax trichodon	shrub	S	2.5 ± 0.2	9	0.01	37.8 ± 53.2	Jul, **Aug**	0.437	Su	340	340	1.00
Ebenaceae												
Diospyros lotus	high tree	D	7.8 ± 0.4[a]	2	0.00	1.0 ± 0.0	**Nov**	—	F	2	2	1.00
Solanaceae												
Tubocapsicum anomalum	herbaceous	S	1.3 ± 0.1	41	0.03	47.8 ± 92.8	Mar, **Sep**, Oct, **Nov**, Dec	0.595	F	1994	1960	0.98
Caprifoliaceae												
Viburnum dilatum	herbaceous	S	3.3 ± 0.4	236	0.18	65.2 ± 92.2	Mar, May, Oct, **Nov**, Dec, Jan, Feb	**< 0.001**	F	15945	15387	0.97
Gramineae												
*Zoysia japonica**	herbaceous	C	1.3 ± 0.2	17	0.01	14.5 ± 19.4	**May**, **Aug**, Oct, Nov	0.300	Sp, Su	317	247	0.78
*Oplismenus undulatifolius**	herbaceous	S	1.5	8	0.01	1.1 ± 0.4	Mar, Oct, **Nov**	0.435	F	10	9	0.89
*Digitaria ciliaris**	herbaceous	S	1.2 ± 0.1[a]	1	0.00	1.0	**Aug**	—	Su	1	1	1.00
Gramineae 2*	herbaceous	S	0.8 ± 0.1	29	0.02	454.9 ± 449.5	**Jun**	—	Su	13192	13192	1.00
Gramineae 1*	herbaceous	S	2.4 ± 0.3	16	0.01	14.1 ± 15.8	**Jun**	—	Su	226	226	1.00
Gramineae 3*	herbaceous	S	1.4 ± 0.4	2	0.00	17.0 ± 1.4	**Jun**	—	Su	34	34	1.00
Ranunculaceae												
Clematis apiifolia *	vine	C	1.8 ± 0.2	2	0.00	1.0 ± 0.0	**Jun, Oct**	—	Su, F	2	2	1.00
Oxalidaceae												
Oxalis corniculata *	herbaceous	S	0.9 ± 0.1	39	0.03	157.2 ± 178.6	**May, Jun**	**< 0.001**	Sp, Su	6131	6131	1.00
Violaceae												
Viola sp.*	herbaceous	S	0.8 ± 0.1	29	0.02	76.6 ± 92.6	**Jun**	—	Su	2221	2221	1.00
Labiatae												
Perilla frutescens *	herbaceous	C	1.7 ± 0.2	45	0.03	4.0 ± 6.3	Mar, **Oct**, Nov, Dec	0.133	F	209	180	0.86
unidentified	-		-	11	0.01	—	Mar, May, Jul, Aug, Oct, Nov	—	—	—	—	—

DE : dispersal event, *DE*$_r$: rate of dispersal event, *IR* : intact rate, *MCD* : mean cubic diameter of seeds, *SHT* : seed handring technique (C: crunching, D: discarded, S: swallowing).

* non fleshy-fruited species.

[a]: name of each months are abbriviated. Months in which number of intact seeds exceed annual mean are shown in bold.

[b]: *P* - values less than 0.05 are shown in bold.

[c]: F: fall, Sp: spring, Su: summer, W: winter.

Table 3. Monthly changes in numbers of intact seeds and species within single feces defecated by the Japanese macaques on Kinkazan, northern Japan.

Month	# fecal sample	*DE*	# Intact seeds per feces (Mean + SD)			# Species per feces (Mean + SD)		
Spring								
March	374	83	7.4	±	46.7	1.5	±	1.3
April	10	1		-			-	
May	158	21	2.3	±	9.8	1.3	±	0.6
Summer								
June	29	29	765.2	±	583.1	4.1	±	1.7
July	56	45	33.1	±	45.2	1.2	±	0.4
August	93	90	75.5	±	67.3	1.3	±	0.5
Fall								
September	76	56	34.3	±	49.5	2.3	±	1.0
October	177	169	43.3	±	67.1	2.9	±	1.5
November	134	130	162.5	±	183.5	2.9	±	1.2
Winter								
December	39	37	196.2	±	354.4	2.6	±	1.0
January	129	82	11.2	±	26.9	2.6	±	1.3
Feburuary	19	4	1.1	±	2.8	1.3	±	0.5
Annual mean	1294	747	58.2	±	175.6	2.0	±	0.9

DE : dispersal event

Table

Table 4. Regional variation in characteristics of seeds detected from feces of Japanese macaques.

Study site	Yakushima	Kashima	Kinkazan	Shimokita
Climate zone	W	C	C	C
Sampling period	1995-2004	2000-2001	2000-2009	2000-2001
# Fecal samples	132	107	1294	75
DE (*DE*$_r$)	131（0.99）	99 (0.93)	747（0.58）	59 (0.79)
# identified plant species	33	18	35	12
Life-form composition (%)				
High trees	13 (38.2)	3 (16.7)	11 (30.6)	1 (8.3)
Shrubs	11 (32.4)	7 (38.9)	5 (13.9)	3 (25.0)
Vines	9 (26.5)	6 (33.3)	5 (13.9)	7 (58.3)
Hemi-parasites	0 (0.0)	0 (0.0)	1 (2.8)	0 (0.0)
Herbaceous plants	0 (0.0)	2 (11.1)	13 (36.1)	1 (8.3)
# Freshy-fruit species (%)	31 (91.2)	16 (88.9)	20 (55.6)	11 (91.7)
MCD (Mean±SD) (mm)	2.5±1.7 (N=12)	2.7±1.2 (N=11)	3.0±2.0 (N=35)	2.3±1.1 (N=15)
Range	1.1-6.4	1.2-4.1	0.8-10.8	1.2-4.1
# species (Mean±SD)	2.8±1.4 (N=131)	2.4±1.5 (N=99)	2.0±0.9 (N=747)	1.9±0.8 (N=59)
References	Otani (2005)	Otani (2003)	This study	Otani (2003)

C: cool temperate zone, W: warm temperate zone, *DE* : dispersal event, *DE*$_r$: rate of dispersal event,
MCD : mean cubic diameter of seeds.